

PATENTS

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Benjamin E. Reubinoﬀ, et al.

Examiner: TO BE ASSIGNED

Serial No.: TO BE ASSIGNED

Art Unit: TO BE ASSIGNED

Filed: Herewith

Docket: 13164a

For: EMBRYONIC STEM CELLS

Dated: March 5, 2002

Assistant Commissioner for Patents
United States Patent and Trademark Office
Washington, D.C. 20231

PRELIMINARY AMENDMENT

Sir:

In connection with the filing of the above-identified application, kindly enter the following preliminary amendments.

IN THE SPECIFICATION:

Please insert the following paragraph at page 1, line 2:

--CROSS-REFERENCE TO RELATED APPLICATION

This application is a divisional of U.S. Serial No. 09/436,164, filed on November 9, 1999.--

CERTIFICATE OF MAILING BY "EXPRESS MAIL"

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Date of Deposit: **March 5, 2002**

I hereby certify that this Preliminary Amendment is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 C.F.R. §1.10 on the date indicated above and is addressed to the Assistant Commissioner of Patents and Trademarks, Washington, DC 20231.

Dated: **March 5, 2002**

Michelle Mustafa

Please replace the paragraph beginning at page 31, line 18 with the following paragraph:

--To monitor expression of Oct-4, RT-PCR was carried out on colonies consisting predominantly of stem cells, or colonies which had undergone spontaneous differentiation as described below. mRNA was isolated on magnetic beads (Dynal AS, Oslo) following cell lysis according to the manufacturer's instructions, and solid-phase first strand cDNA synthesis was performed using Superscript II reverse transcriptase (Life Technologies). OCT-4 transcripts were assayed using the following primers: 5'-CGTTCTCTTTGGAAAGGTGTTC (forward) (SEQ ID NO: 1) and 3'-ACACTCGGACCACGTCTTTC (reverse) (SEQ ID NO: 2). As a control for mRNA quality, betaactin transcripts were assayed using the same RT-PCR and the following primers: 5'-CGCACCCTGGCATTGTCAT-3' (forward) (SEQ ID NO: 3), 5'-TTCTCCTTGATGTCACGCAC-3' (reverse) (SEQ ID NO: 4). Products were analyzed on a 1.5% agarose gel and visualized by ethidium bromide staining.--

Please replace the paragraph beginning at page 32, line 19 with the following paragraph:

--Clusters of cells destined to give rise to neural precursors were identified by their characteristic morphological features in central areas of ES cell colonies 2-3 weeks after plating. The clusters were dissected mechanically by a micropipette and replated in fresh serum free medium. Within 24 hours they formed spherical structures. The expression of the transcription factor PAX-6 and the intermediate filament nestin by these clusters was demonstrated by RT-PCR as described above. The following primers were used for PAX-6 and nestin respectively: Pax-6 forward primer, 5'AACAGACACAGCCCTCACAAACA3' (SEQ ID NO: 5); Pax-6 reverse primer, 5'CGGGAAGTTGAACTGGAAGTAC3'(SEQ ID NO: 6); nestin forward primer, 5'CAGCTGGCGCACCTCAAGATG3' (SEQ ID NO: 7); nestin reverse primer, 5'AGGGAAGTTGGGCTCAGGACTGG3' (SEQ ID NO: 8).--

IN THE CLAIMS:

Please cancel claims 1-32 and 35 without prejudice.

REMARKS

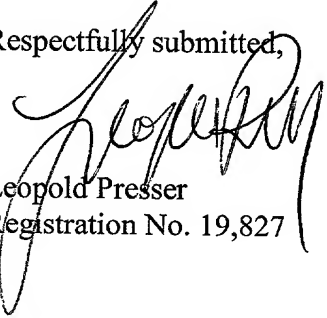
The specification has been amended to add the cross-reference to the parent application. The specification has also been amended to insert the sequence identifiers. No new matter is introduced.

Claims 1-32 and 35 have been canceled without prejudice as drawn to non-elected embodiments. Applicants reserve the right to pursue the subject matter of the canceled claims in one or more divisional applications.

Attached hereto is a marked-up copy of the amendment to the claims, captioned "Version with Markings to Show Changes Made."

It is respectfully submitted that the present case is in condition for examination on merits, which action is earnestly solicited.

Respectfully submitted,


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Enclosure: Version with Markings to Show Changes Made

Serial No.: TO BE ASSIGNED

Version with Markings to Show Changes Made

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In the Claims:

Please cancel claims 1-32 and 35 without prejudice.